

Page 7, line 21, after "assay." please insert the following:

--and

Figure 5 is a graph showing the detection of DNA and white blood cells on FTA-NC membranes having a 1.2 μm pore size using ELISA on basis antibodies to human DNA.--

Please insert the following on Page 40 , Line 11:

-EXAMPLE 7:

The following experiment demonstrates that DNA or genetic material isolated from low amounts of white blood cells, such as 33 cells per well or .33 cell/ μl , can be measured.

White blood cell concentration of 1 cell per mL represents the amount of cells allowed for Whole blood unit, having a volume of 500 mL , to be marked as leukoreduction (LR) according to the European Standard. This method is able to detect such low levels of concentrations that is can be recommended for QC of leukoreduced blood.

Materials and Protocol:

White blood cell suspensions were obtained from whole blood samples after lysing red blood cells with ammonium chloride buffer. The concentration of white blood cells can be in the range of 1 to 1000 cells per μl . In this experiment, 100 mL of white blood cells from leukoreduced blood was loaded on FTA membrane and resulted in the collection of 600 pg DNA per well (note that there is 6 pg of DNA in a single human cell). Fluorescent stain of cell nucleus with

Propidium Iodine detergent solution and fluorescent microscopy was used for validation of white blood cell lysis on the FTA membrane.

A 96 well plate with control nitrocellulose membrane and the FTA membrane were loaded with white blood cells. Two methods were used. At first, the white blood cell suspension was spotted on the membrane in a volume of 2.5 μ l/well. According to a second protocol, different volumes of white blood cells in the range of 20 – 180 μ l/well were applied to the membrane by vacuum filtration. Two different ELISA systems were used to measure DNA in each well. The first ELISA was based on polyethyleneimine-peroxidase conjugate. The second protocol used monoclonal antibodies specific to human genomic DNA.

The detection limit was achieved by conducting an experiment when the same plate was loaded with genomic DNA samples in concentration range of 0.2 – 20 ng per well and white blood cell samples in amount of 90 – 360 per well. DNA samples were spotted on the membrane in volume of 2 mkl per well. White blood cell samples were loaded in volumes of 30 – 120 mkl per well by vacuum filtration.

Results:

The white blood cells stained with Propidium Iodine and spotted on nitrocellulose membrane can be observed with a fluorescent microscope which are seen as bright pink spots spread over the membrane surface. The amount of cells counted on the membrane depends on the amount of white blood cells loaded. Propidium Iodine staining indicated that no intact cells remained on the FTA membrane spotted with the same amount of white blood cells. The color intensity of the ELISA product developed on the FTA membrane has a linear correlation with the amount of white blood cells loaded per well. The detection limit for white blood cell was determined to be 100 cells per well. This represents

the concentration of 1 cell/ μ l. The volume capacity was found as 100 μ l of white blood cells suspension per well. When 3'3-diaminobenzidine was used as substrate for peroxidase, an insoluble product formed in the white blood cell positive wells as brown rings on the FTA membrane. The thickness and intensity of the rings depends on the amount of cells in the well.

The volume capacity for 0.8 – 1.2 FTA-NC membrane was shown to be ~100 mkl of white blood cell suspension per well. So, it is possible to obtain 600 pg of DNA per well. This amount can be detected on FTA-NC membranes with ELISA using antibodies specific to human DNA above the background.

The sensitivity of the method, which causes the FTA membrane to lyse cells and capture of cell DNA and ELISA detection system on the basis of antibodies specific to human DNA is 0.2 ng of DNA per well in a 96 well plate format. In the initial experiments the sensitivity of the method was found as ~100 mkl per well of white blood cell suspension with cell concentration of 3 cell per mkl (as average count), or 5 +/- 4 cells/ mkl. Accuracy at this level is in the range of 5 +/- 4 cells per mkl. However, in an additional experiment, using the same method, the sensitivity was determined to be 33 cells per well.

With ELISA, on the basis of antibodies specific to human DNA, it was possible to see a difference between control wells loaded with PBS and DNA and white blood cell positive wells loaded with 0.2 ng DNA/well or 60 mkl white blood cells/well. There is a linear dependency between color intensity of the assay and the amount of DNA. Additionally, there is a linear dependency between the assay and the white blood cells loaded per well. The data is presented in Figure 5.